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Optimization of fucoidan extraction from brown algae (*Sargassum swartzii*) using response surface methodology and assessment of its antioxidant activity

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Abstract

Fucoidan is among the sulfated polysaccharides that originate from the cell walls of brown algae. It is recognized as the most vital factor in pharmaceuticals and food due to its many beneficial properties, such as anticancer, anti-inflammatory, antioxidant, etc. Applying modern extraction techniques and optimizing extraction conditions have recently received special attention in order to achieve high extraction efficiency while being environmentally and health-friendly. This study used the Response Surface Methodology (RSM) to optimize the extraction conditions of fucoidan from the brown algae *Sargassum swartzii* collected from Nam Du archipelago with the support of ultrasound. The effects of various temperatures, including 60°C, 70°C, 80°C (A), and duration of time 30, 45, 60 min (B) were studied through the central composite design model. The antioxidant activity of fucoidan extracted under optimal conditions was also investigated. The tests revealed that the optimal conditions for fucoidan extraction were at 75°C for 50 min, corresponding to the extraction efficiency (crude fucoidan content) of 2.59%; the sulfate and total polyphenol contents were 29.11% and 1.87%, respectively. The analysis of the structural characteristics of fucoidan was taken using Fourier-transform infrared spectroscopy (FT-IR). In addition, fucoidan from *Sargassum swartzii* exhibited biological activity in antioxidant properties, DPPH and ABTS free radical scavenging efficiency of 51.04% at 1.250 mg/mL and 72.47% at 0.5 mg/mL, respectively. These findings can be applied to improve the field of pharmaceuticals and functional food.

Keywords: Antioxidant activity, fucoidan, *Sargassum swartzii*, RSM

1. Introduction

Nam Du archipelago is located in the Gulf of Thailand and belongs to Kien Giang province. This area is known to be rich in natural resources, with ideal natural conditions for many species of organisms, including seaweed. There are 43 species of seaweed potential for use with high value in Nam Du archipelago. If calculated on a distribution area of 90 ha, *Sargassum* is one of the dominant seaweed genera that contributes the most to the biomass of seaweed resources in Nam Du archipelago (72.0 ± 55.3 g/m²). In particular, *Sargassum swartzii* is a species of the genus *Sargassum* with high yield, widely distributed in Nam Du archipelago, bringing many economic and pharmaceutical values [1]

Seaweed has been considered a source of polysaccharides containing many valuable natural bioactivities for many years. Polysaccharides can be sulfated or non-sulfated [2]. Fucoidan is a sulfated polysaccharide mainly found in brown algae, consisting of a fucopyranose chain and some monosaccharides. It has been suggested to have many biological activities such as anticancer, antioxidant, immunomodulatory, antiviral, antithrombotic, and

anti-inflammatory, thus may benefit human health [3]. Many attempts at the extraction method and activity of fucoidan extracted from brown algae have been made in Vietnam. However, these studies mostly used conventional chemical methods, which may affect their contents and natural structures and thus affect their biological activity. Therefore, extraction methods for green fucoidan have been developed, including microwave-assisted extraction (MAE), ultrasound-assisted extraction (UAE), and enzyme-assisted extraction (EAE) [3]. Microwave-assisted extraction (MAE) is performed by heating seaweed with electromagnetic waves at frequencies ranging from 300 to 3000 MHz [4, 5]. However, thermally unstable compounds may decompose [6, 7]. Ultrasound-assisted extraction (UAE) is a cheap, fast, and efficient method that uses ultrasound to enhance extraction. In addition, this method can be applied in industrial environments [6, 8, 9]. UAE can be performed at room temperature [10]. However, the disadvantage of UAE is that the noise from ultrasound is very annoying. Enzyme-assisted extraction (EAE) is a green method with many benefits such as it degrades the cell wall without affecting the internal components of the seaweed [11]. Another study has shown that EBE shortens the extraction time; increases the extractability; preserves the bioactivity of fucoidan and reduces or eliminates the use of solvents. Besides, EAE is environmentally friendly; renewable, relatively cheaper than organic solvents; and exhibits high specificity [6, 12, 13]. On the contrary, it also has some disadvantages such as the possibility of enzyme denaturation at high temperatures [6, 14]. In addition, enzyme extraction of fucoidan requires prolonged purification because polyphenols are released as by-products that contaminate fucan [15, 16]. In summary, UAE not only gives high extraction efficiency but also shortens the extraction time and temperature, while still preserving the molecular structure and biological activity of fucoidan. Therefore, we chose UAE as an optimal and more suitable extraction method for this study.

Fucoidan extraction conditions are also of interest to many researchers because these factors directly affect fucoidan extraction efficiency, structure, chemical composition, and biological activity. Various approaches have been proposed to optimize the extraction conditions. However, the Response Surface Methodology (RSM) is a widely used optimization method. Compared with the conventional optimization method (optimizing each factor), RSM has the outstanding advantage of saving time and considering the interactions between variables, helping the results obtained be more reliable [17]. Previous studies in Vietnam have focused on the traditional method of extracting fucoidan. However, this method can change the structure and reduce the biological activity of fucoidan. This study was in an effort to optimize the conditions for ultrasound-assisted fucoidan extraction (UAE) and investigate the antioxidant property of fucoidan from *Sargassum swartzii* collected in Nam Du archipelago, Kien Giang province, Vietnam, aiming to improve the greener and more efficient extraction process, contributing to the discovery and effective use of economic resources for seaweeds in the Southwest region.

2. Materials and methods

2.1 Sample collection

Brown algae *S. swartzii* was collected in April 2024 in Nam Du archipelago of Kien Giang province. The sample was identified by Dr. Nguyen Thi Kim Hue, Department of Biology, Faculty of Natural Sciences, Can Tho University. The algae were washed with tap water, dried in a hot air oven at 50°C, crushed, and stored at room temperature. All samples were ground under the same conditions and particle size was not measured or varied in this study.

2.2 Chemicals

The chemicals used in the experiment included: ABTS2,2'-Azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) (Merck), Folin-Ciocalteu reagent (Merck), 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma), bovine serum albumin (Himedia), gallic acid (Sigma) and some other chemicals at analytical grade.

2.3 Extraction of crude fucoidan from *S. swartzii* (SSF)

The extraction of fucoidan from algae was performed as follows: 10 g of algae powder was treated by soaking overnight with ethanol 96°. Then, it was incubated with 80° ethanol at 70°C for 4 hours to remove pigments and compounds with low molecular weight. After washing and filtering, the residue was added to water at a 1:30 (w/v) ratio and placed in an ultrasonic bath with changes in two temperature and time extraction conditions. After centrifugation and filtration, the extract was mixed with 2% CaCl₂ solution and kept overnight at 4°C. Remove the alginate precipitate and then add ethanol 96° at a ratio of 1:1 (v/v) before keeping overnight at 4°C. After centrifugation, the crude fucoidan residue was dried at 50°C to a constant mass and weighed. The extracted crude fucoidan content was calculated based on the dry mass of algae [18]. The experiment to optimize the extraction

conditions for SSF using the UAE method was designed according to a two-factor central composite design (CCD). The influencing factors investigated included temperature (A: 60-80°C) and time (B: 30-60 min), with the objective function being the crude fucoidan content Y. The levels of independent variables are encoded according to Table 1.

Table 1 Factors and levels of optimization of SSF extraction conditions used in UAE method.

Factors	Sign	Encryption level				
		$-\alpha(-1.41)$	-1	0	+1	$+\alpha(+1.41)$
Temperature (°C)	A	55.9	60	70	80	84.1
Time (min)	B	23.8	30	45	60	66.2

Response Surface Methodology (RSM) based on a rotatable Central Composite Design (CCD) was applied to optimize the extraction variables. Each factor was coded at five levels: $-\alpha$ (-1.41), -1 (low), 0 (center), $+1$ (high), and $+\alpha$ ($+1.41$), where $\alpha = 1.414$ to ensure rotatability of the design. The total number of experimental runs was 14, including four factorial points ($\pm 1, \pm 1$), four axial points ($\pm \alpha, 0$ and $0, \pm \alpha$), and six replications at the center point to estimate pure error and assess model adequacy (Table 2). The mathematical model representing the dependence of extraction efficiency and coding factor is a quadratic polynomial equation of the form:

$$Y = \beta_0 + \beta_1 A + \beta_2 B + \beta_{12} AB + \beta_{11} A^2 + \beta_{22} B^2 \quad (1)$$

Where: Y is the predicted crude fucoidan content; β_0 is the intercept; β_1 and β_2 are the linear coefficients; β_{12} is the interaction coefficient; and β_{11} and β_{22} are the quadratic coefficients of the independent variables A (temperature) and B (time), respectively.

2.4 Chemical analysis methods

FT-IR spectrum: Fucoidan was confirmed through the FT-IR infrared spectrum, measured at the Chemistry Department, College of Natural Sciences by FT/IR spectrometer Jasco 4600 (Japan).

Total polyphenol content (TPC) was identified by the colorimetric method using the Folin Ciocalteu reagent [19] specifically as follows: 1 mL of sample, gallic acid standard, or blank was mixed with 70 mL of water and Folin–Ciocalteu reagent, incubated for a few min at room temperature, then treated with sodium carbonate and diluted to 100 mL. After incubation for 2 h at room temperature, the absorbance was measured at 765 nm against a blank. A calibration curve prepared with gallic acid was used, and the results were expressed as gallic acid equivalents (GAE, mg/L).

The content of sulfate was analyzed using the BaCl_2 – gelatin method [20] as follows: After acid hydrolysis, the liberated sulfate ions reacted with 1 mL of BaCl_2 –gelatin reagent to form BaSO_4 , which was measured spectrophotometrically at 360 nm against a standard curve prepared with potassium sulfate.

2.5 DPPH free radical scavenging assay

The calculation of the antioxidant properties of fucoidan extract was conducted using the following protocol. 100 μL DPPH (6×10^{-4} M) (Sigma, Germany) was combined with 100 μL fucoidan extract at different concentrations (0.039; 0.078; 0.156; 0.313; 0.625; and 1.250 mg/mL). The test solution was stirred thoroughly before leaving in the dark at room temperature for 60 min. Measuring the mixture absorbance was taken at 517 nm. The DPPH radical reducing ability was determined by the decrease in absorbance. The 50% inhibitory concentration (IC_{50}) was estimated using the standard curve $y = ax + b$. A lower IC_{50} value indicated a higher antioxidant capacity [21].

2.6 ABTS radical scavenging assay

The method of ABTS radical scavenging followed the procedure: the ABTS reagent (Sigma, Germany) was prepared by combining 5 mL of ABTS 7 mM with 88 μL of 140 mM potassium persulfate. The reagent was then incubated in the dark at room temperature for 16 h. After being treated on a spectrophotometer to achieve an optical density of 0.7 ± 0.05 (1:44, v/v) at 734 nm, the $\text{ABTS}^{\cdot+}$ free radical neutralization activity was performed by adding 990 μL $\text{ABTS}^{\cdot+}$ to 10 μL of fucoidan extract solution at concentrations of 0.031; 0.063; 0.125; 0.25; 0.50 mg/mL. The resulting mixture was kept for 6 min before measuring the absorbance at 734 nm [22].

$$E (\%) = (Absc - Absm)/Absc \times 100$$

Where:

E: Antioxidant activity (ABTS free radical scavenging ability (%))

Absc: Absorbance value of the control sample

Absm: Absorbance value of the test sample

2.7 Statistical analysis of data

The experiments were completed after three times. The data were displayed as mean $\pm$ standard deviation (mean $\pm$ SD). They were calculated and graphed using Microsoft Excel 2019. The experiment was designed according to the central composite design model, and statistical analysis was processed using STATGRAPHICS Centurion XV software, version 15.1.02 (StatPoint Technologies Inc. Warrenton, VA, USA). One-way analysis of variance (ANOVA) and LSD test were used to determine the difference. P values < 0.05 were seen as significant.

3. Results and discussion

3.1 Results of optimizing the extraction conditions

The crude fucoidan content extracted in the experiments ranged from 1.89% to 2.63% (Table 2). At the central point (temperature of 70°C, extraction time of 45 min), the maximum content was 2.63%.

Table 2 Experimental design matrix and observed responses according to the two-factor Central Composite Design (CCD) of optimizing of crude fucoidan extraction from *S. swartzii*.

Experiment	Temperature (°C)	Time (min)	Fucoidan content (% dried algae)
1	55.9	45	2.04
			2.05
			2.02
2	60	30	1.91
			1.89
			1.92
3	60	60	2.25
			2.25
			2.24
4	70	23.8	2.05
			2.04
			2.05
5	70	45	2.54
			2.38
			2.63
6	70	45	2.38
			2.63
			2.54
7	70	45	2.54
			2.59
			2.46
8	70	45	2.56
			2.60
			2.59
9	70	45	2.54
			2.54
			2.54
10	70	45	2.57
			2.59
			2.46
11	70	66.2	2.41
			2.40
			2.39
12	80	30	2.37
			2.37
			2.36
13	80	60	2.54
			2.53
			2.52
14	84.1	45	2.51
			2.50
			2.52

The effects of dependent variables on SSF extraction are presented as a quadratic model (Equation 2).

$$\text{Fucoidan} = -7,7167 + 0,2118A + 0,0904B - 0,0012A^2 - 0,00029*AB - 0,000683B^2 \quad (2)$$

Where: A is temperature, B is time

The results of the ANOVA analysis of independent variables in the correlation model between crude fucoidan content extracted from *S. swartzii* with extraction temperature and time are shown in Table 3. The values of linear factors (A, B), AB interaction, and quadratic values AA and BB all affect the extraction capacity, showing a high level of reliability ($P < 0.05$). Furthermore, the lack-of-fit of the model with P value = 0.7634 ($P > 0.05$) shows that the model is suitable for predicting crude fucoidan content in the ultrasonic-assisted extraction process.

Table 3 Results of analysis of variance and model fit for crude fucoidan content extracted from *S. swartzii*.

Factor	SS	Df	MS	F-Ratio	P-Value
A: Temperature	0.74854	1	0.74854	262.10	0.0000
B: Time	0.377271	1	0.377271	132.10	0.0000
AA	0.366445	1	0.366445	128.31	0.0000
AB	0.0234083	1	0.0234083	8.20	0.0072
BB	0.521987	1	0.521987	182.78	0.0000
Lack-of-fit	0.00331193	3	0.00110398	0.39	0.7634
Pure error	0.0942444	33	0.00285589		
Total (corr.)	2.07416	41			

$R^2 = 95.30\%$ $R^2(\text{adj}) = 94.64\%$. Standard Error of Est. = 0.0534405

Where: SS means to the sum of squares; DF means the degrees of freedom; MS refers to the mean square.

The suitability of the model was also checked through the correlation coefficient of determination R^2 . The model is well correlated when the correlation coefficient of determination $R^2 > 0.8$ (Guan & Yao, 2008). The correlation model built from the experiment satisfied the condition with $R^2 = 0.9530$, indicating that the change in crude fucoidan content can be predicted by the model (Equation 1). The Pareto chart (Fig. 1) proves that the linear factor values (A, B) have positive effects, and the interaction values (AB) and quadratic (AA, BB) have negative effects on the SSF extraction process. All bars crossing the vertical line are statistically significant at the 95% level ($P < 0.05$).

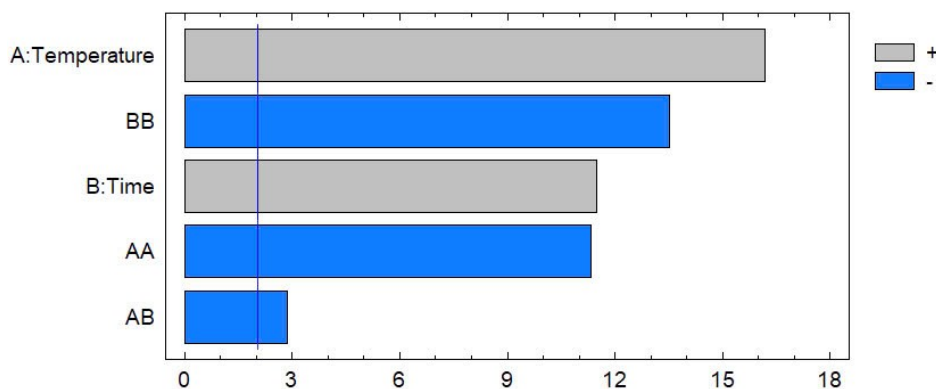


Figure 1 Pareto chart of the influence of independent variables on crude fucoidan during ultrasound-assisted extraction.

The response surface of fucoidan content to the effects of temperature and time is depicted in Fig. 2a. The results indicate that when the time is extended, the contact between the solvent and the raw material will increase, making it easier for fucoidan to migrate into the environment. However, if the time is too long, the crude fucoidan content in the extract tends to balance. Increasing the temperature can also create a higher mass transfer rate and solvent diffusion while reducing the surface tension and viscosity, causing more polysaccharides to dissolve in the extraction medium [23]. At higher temperatures (80°C), carrageenan and agar in algae begin to be extracted [24]. The high viscosity of these compounds inhibits the extraction of fucoidan [25].

Furthermore, high temperatures lead to the destruction of the natural chemical structure of fucoidans. Increasing the extraction time will facilitate the contact of brown algae powder with the solvent combined with higher ultrasound

waves to help break down brown algae cells, resulting in a greater amount of fucoidan released into the solvent. However, an extraction time that is too long (over 50 min) combined with too high ultrasound power will reduce the quality of fucoidan. Some studies have also shown that prolonging the extraction time reduces the extraction efficiency of fucoidan from brown algae [26, 27]. Particle size is also an important factor affecting the extraction efficiency. The finer the particle size, the higher the extraction efficiency, thanks to the enhanced solvent permeability and solute diffusion. However, too fine a particle size will increase the solute absorption into the solid and make subsequent filtration difficult [28]. Although this issue was not addressed in this study, future studies should specifically investigate the effect of particle size on fucoidan yield and quality.

The optimal values of the independent variables in the extraction process are determined in Fig. 2b: The maximum point of the objective function Y (fucoidan content) is achieved when the temperature and extraction time are 76.30°C and 49.77 min. At that time, the estimated maximum crude fucoidan was 2.61%. To confirm the results, fucoidan extraction experiments were performed at the optimal conditions: 76°C, 50 min, and the crude fucoidan content was 2.59%. Statistically, no statistical difference was observed between the model-predicted and experimental confirmation results at the 95% confidence interval.

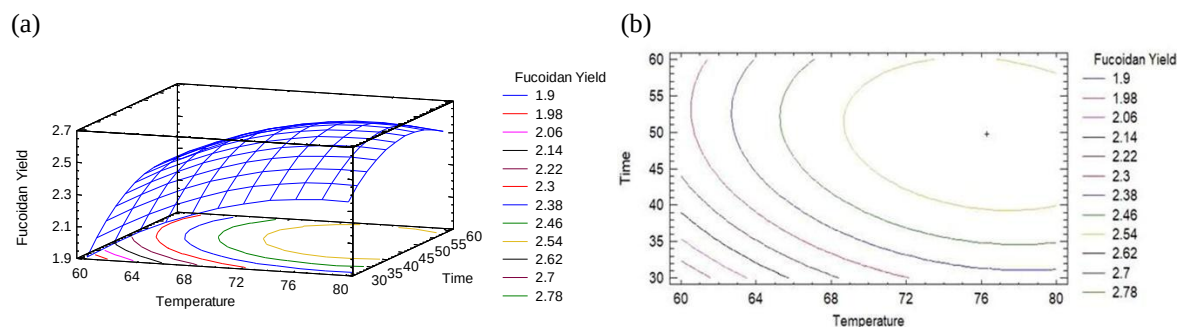


Figure 2 3D (2a) and 2D (2b) response surface models of ultrasound-assisted extraction of crude fucoidan from *S. swartzii*

3.2 Chemical composition of crude fucoidan from *S. swartzii* algae

The sulfate and total polyphenol contents in the extracted crude fucoidan were 29.27 (%) and 1.76 (%), respectively (Table 4). This result is higher compared to the other research results, namely 20.83 (%) and 1.66 (%) for sulfate and polyphenol in the same species *S. swartzii* [29], and lower compared to the total polyphenol content in fucoidan extracted from *S. glaucescens* algae which is 2.7% [30]. The above difference is because the sulfate and total polyphenol contents can vary depending on the species, season, geographical area, and extraction and analysis method [3].

Table 4 Sulphate and total polyphenol (TPC) contents in crude fucoidan from *S. swartzii* algae

Criteria	Content
Sulfate (%)	29.27±0.33
TPC (%)	1.76±0.02

3.3 FT-IR spectrum of fucoidan extracted from *S. swartzii* algae

The functional groups of crude fucoidan extracted from *S. swartzii* under optimized conditions were analyzed by FT-IR spectroscopy and are presented in Fig. 3. The absorption signal at 1253.5 cm^{-1} is characteristic of the vibration of the S=O bond; the signal at the peak of 816.706 cm^{-1} characterizes the vibration of the C-O-S bond; and the signal at the peak of 1036.55 is characteristic of the vibration of the C-O-SO₃ group; this confirms the presence of the sulfate group. In addition, the peak at 3408.57 cm^{-1} characterizes the stretching vibration of the O-H group, and the signal at 1622.8 cm^{-1} is characteristic of the group, representing the symmetric and asymmetric vibration of the carboxylate group (C=O). (Table 5). The absorption signal peaks found above were similar to fucoidan from previously studied species such as *S. ilicifolium* [31], *Sargassum polycystum* [32], *Turbinaria decurrens* [33].

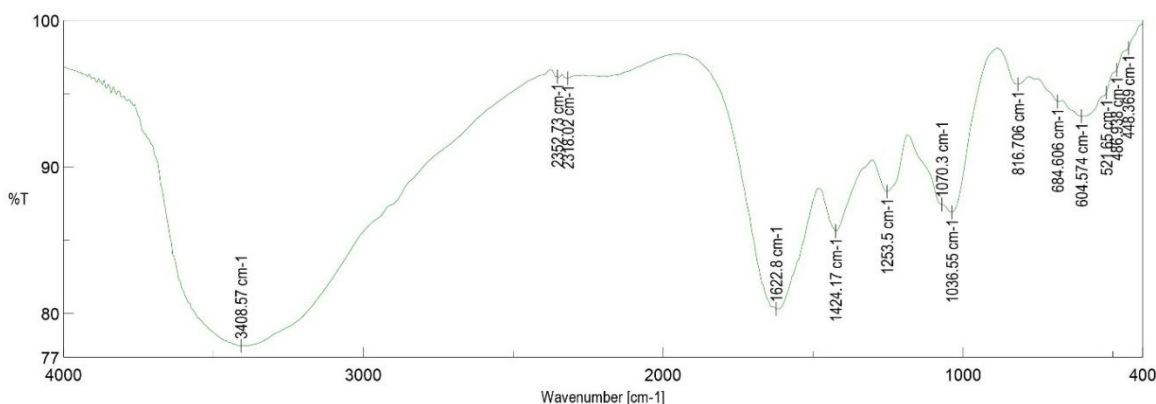


Figure 3 FT-IR spectrum of fucoidan extracted from *S. swartzii*.

Table 5 FT-IR spectrum of fucoidan from *S. swartzii*.

No.	Absorption peak (cm ⁻¹)	Vibration, characteristic functional group
1	3408.57	O-H group stretching vibration
2	1622.8	Symmetric and asymmetric vibrations of C=O group
3	1253.5	S=O group stretching vibration
4	1036.55	C-O-SO ₃ vibration
5	816.706	Vibration of C-O-S bond

3.4 Antioxidant efficiency by DPPH method

The experimental results of evaluating the antioxidant property by the DPPH method in Fig. 4 were the evidence that crude fucoidan from *S. swartzii* had antioxidant potential, the DPPH free radical scavenging efficiency increases linearly with the concentration according to the linear regression equation $y = 42.072x + 2.2025$ ($R^2 = 0.966$), and IC_{50} was 1.14 mg/mL. When the crude fucoidan concentration increased from 0.039 mg/mL to 1.250 mg/mL, the free radical scavenging efficiency increased significantly ($P < 0.05$) from $1.71 \pm 0.25\%$ to $51.04 \pm 2.77\%$.

The IC_{50} value reflects the 50% antioxidant efficiency; the smaller the value, the better the antioxidant activity of the extract [34]. The results of this study showed that the antioxidant capacity of crude fucoidan from *S. swartzii* was higher than that of fucoidan from *D. dichotoma* with IC_{50} of 4.59 mg/mL [35]. It was lower than the antioxidant capacity obtained in *S. ilicifolium* (A03) when extracted at 65 and 80°C at 81 and 78%, respectively, at 1 mg/mL [36]. DPPH free radical scavenging activity is related to the reaction of transferring hydrogen atoms or electrons from sulfate radicals to free radicals. The higher the sulfate content, the higher the antioxidant activity. In addition, the polyphenol content in fucoidan extract is also thought to enhance antioxidant activity [37].

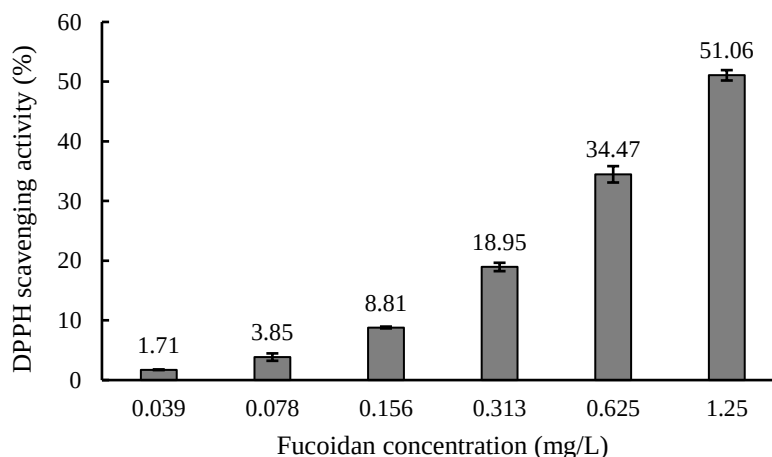


Figure 4 The scavenging activity of DPPH free radicals of SSF

3.5 Antioxidant efficiency by ABTS method

The experimental findings highlighted that the ABTS radical scavenging activity of SSF increased with the increase in fucoidan concentration ($P < 0.05$) (Fig. 5), and the maximum activity was found to be $72.47 \pm 1.31\%$ at 0.5 mg/mL , with a linear regression equation $y = 137.11x + 8.1987$ ($R^2 = 0.9535$).

The crude fucoidan from *S. swartzii* exhibited a strong antioxidant capacity when neutralizing 50% of ABTS free radicals (IC_{50}) at 0.30 mg/mL . This result showed that the antioxidant efficiency of SSF was higher when compared to fucoidan from *D. dichotoma* with an IC_{50} of 8.55 mg/mL [35]. In addition, according to the other study, the IC_{50} values of polysaccharides from brown algae (*Padina gymnospora*), red algae (*Kappaphycus alvarezii*) and green algae (*Kappaphycus striatus*) were $2.565 \pm 0.02 \text{ mg/mL}$, $7.859 \pm 0.09 \text{ mg/mL}$ and $15.56 \pm 0.12 \text{ mg/mL}$, respectively [38].

The free radical scavenging ability of ATBS is related to the existence of a reducing agent, which has been shown to have an antioxidant property by breaking the free radical chain through donating a hydrogen atom. In general, the antioxidant activity of marine polysaccharides may be due to various factors, such as structure, molecular weight, degree of sulphation, sugar type, and location. These factors are influenced by harvest time, season of the year, geographical location, extraction and pretreatment procedures, etc. [3].

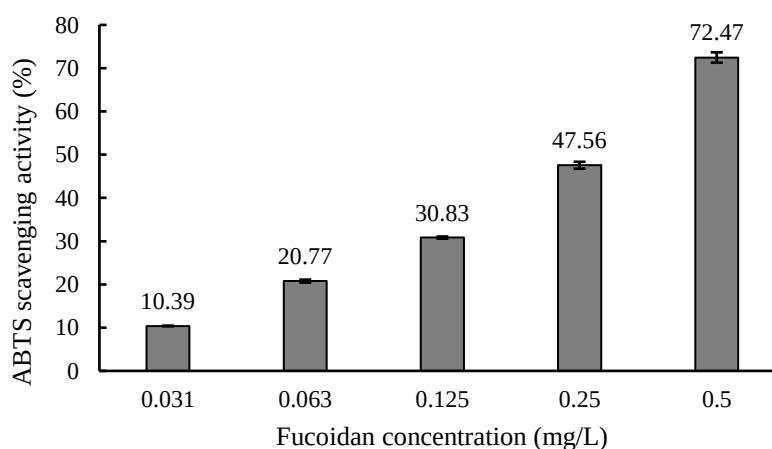


Figure 5 The scavenging activity of ABTS free radicals of SSF

4. Conclusion

In this study, the optimal conditions for fucoidan extraction from brown algae *Sargassum swartzii* by ultrasonic-assisted extraction were investigated to obtain fucoidan with an experimental content of 2.59% (calculated on the dry weight of algae) at 75°C in 50 min, which was close to the theoretical estimate of 2.61%. Analysis of variance proved that the regression model was completely accurate and suitable for optimizing the conditions for fucoidan extraction from brown algae by ultrasonic-assisted extraction. The DPPH and ATBS free radical scavenging abilities of the extracted fucoidan also showed potential antioxidant capacity with IC_{50} values of 1.14 mg/mL and 0.30 mg/mL , respectively. These findings suggest that fucoidan from *Sargassum swartzii* has potential applications in pharmaceuticals. The ultrasound-assisted extraction (UAE) process can be scaled up to industrial scale. Future studies should focus on purification, structural characterization, and in vivo evaluation to confirm biological activity and safety.

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6. Author contributions

NNH and MTD: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft; HHP and PTNN: Validation, Writing –review & editing; TTM: Supervision.

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